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Development and Validation of a RP- HPLC Method for Simultaneous Estimation of Rosiglitazone and Metformin in Bulk and Tablet Dosage Form

Dhirender Singh Mittan[a*], Suresh C. Dwivedi[a], Ashok Kushnoor[b]

[a] School of Pharmacy, Suresh Gyan Vihar University, Jaipur, Rajasthan, India.
[b] Shri Gopichand College of Pharmacy, Baghpat, Uttar Pradesh, India.

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Abstract

A simple reversed-phase high-performance liquid chromatographic (RP-HPLC) method has been developed and validated for simultaneous determination of rosiglitazone and metformin in bulk and tablet dosage form. Chromatographic analysis was performed on a C_{18} column (250x 4.6 mm, 5µm) with a mixture of Ammonium dihydrogen Phosphate buffer (pH 4.5): Acetonitrilein in the ratio 65:35 as mobile phase, at a flow rate of 1.0 mL min^{-1}. UV detection was performed at 230 nm. The method was validated for accuracy, precision, specificity, linearity and sensitivity. The retention times of rosiglitazone and metformin were 7.19±0.044 and 5.57±0.038 min, respectively. Calibration plots were linear over the concentration ranges 12–32 µg mL^{-1} and 20–70 µg mL^{-1} for rosiglitazone and metformin, respectively. The Limit of detection was 1.100 and 0.712 µg mL^{-1} and the quantification limit was 3.66 µg mL^{-1} and 2.41 µg mL^{-1} for metformin and rosiglitazone, respectively. The accuracy of the proposed method was determined by recovery studies and found to be 97.72% to 100.46%. Commercial tablet formulation was successfully analyzed using the developed method and the proposed method is applicable to routine analysis of determination of rosiglitazone and metformin in bulk and tablet dosage form.

Keywords:

Rosiglitazone, Metformin, RP-HPLC, ICH guidelines

1. Introduction

Rosiglitazone is a thiazolidinedione derivative and it is used for the treatment of type 2 diabetes mellitus, chemically it is 5-[[4-[2-(5-ethylpyridin-2-yl) ethoxy]phenyl]methyl]-1,3-thiazolidine-2,4-dione. Rosiglitazone is an oral antidiabetic agent and acts as an agonist at PPAR gamma receptors have acts primarily enhances tissue sensitivity to insulin. Metformin is an antihyperglycemic agent, which improves glucose tolerance in patients, chemically it is 3-(diaminomethylidene)-1,1- dimethylguanidine. Metformin is used for the treatment of with type 2 diabetes, lowering both basal and postprandial plasma glucose. Metformin decreases hepatic glucose production, decreases intestinal absorption of glucose, and improves insulin sensitivity by increasing peripheral glucose uptake and utilization. A literature survey reveals that various analytical methods like rosiglitazone by HPLC and MECK [1], Simple HPLC method for the determination of rosiglitazone in human plasma [2], metformin in human plasma using ion-pair HPLC [8] Simultaneous HPLC estimation of metformin in combination

* **Corresponding Author**
E-mail: chdsmittan@gmail.com
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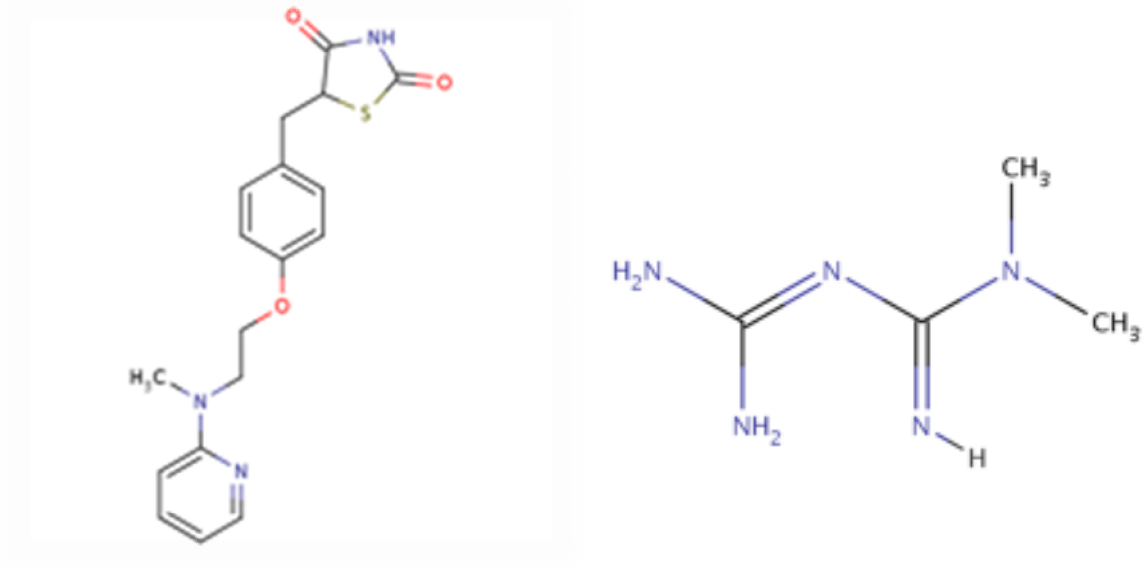
with rosiglitazone [3], rosiglitazone with gliclazide in tablet [4], rosiglitazone and gemfibrozil in human plasma [5]. Simultaneous LC-UV estimation of rosigliazone and glimepiride in plasma [6], rosigliazone and glimepiride in human plasma [7].

But these methods are sophisticated,expensive and time consuming when compared to simple HPLC method. There is need for a interest to develop simple, accurate, specific, sensitive, precise and reproduciable simultaneous HPLC method for the estimation of rosiglitazone and metformin in bulk and its formulation.



(a) (b)

Fig. 1. Chemical structure of Rosiglitazone (a) and Metformin (b)

2. Experimental

2.1. Materials and Methods

Pure standard of rosiglitazone and metformin (Assigned purity 99.98%) was obtained as a gift sample from Micro labs Pvt. Ltd, Badi, India. The gift samples were used as standard without further purification. HPLC grade water, methanol (Qualigens), ammonium dihydrogen phosphate, glacial acetic acid and ammonia (S.D. fine chemicals, Mumbai, India), were used throughout the experiment. Commercial pharmaceutical preparation (Avandamet & Rosimet) which was claimed to contain 500mg of metformin and 2 mg of rosiglitazone is used in analysis. The chemical structure and purity of the sample obtained was confirmed by TLC, IR, Melting point studies.

2.2. Instrumentation and Chromatographic Conditions

High performance liquid chromatograph, Shimadzu pumpLC-10AT VP equipped with universal injector (Hamilton 25 µL) SPD10A, UV-VIS detector SPD10A-10A VP (Shimadzu) was used. Isocratic elution of mobile phase comprising of Ammonium dihydrogen phosphate buffer 65% (pH 4.5) Acetonitrile 35% at flow rate of 1.0 ml min^{-1} was performed on C_{18} column (250x 4.6 mm, 5µm). The effluent was detected at 230 nm. The retention times of rosiglitazone and metformin were 7.19±0.044 and 5.57±0.038 min. The column temperature was maintained at ambient and the volume of injection was 20 µL. Prior to injection of analyte, the column was equilibrated for 30- 40 min with mobile phase.

2.3. Preparation of mobile phase

The HPLC grade solvents were used for the preparation of mobile phase, isocratic elution of mobile phase comprising of of Ammonium dihydrogen phosphate buffer 65% (pH 4.5) Acetonitrile 35% [(Solvent A), Ammonium dihydrogen Phosphate Buffer: Dissolve 2.003 gm of ammonium dihydrogen phosphate (0.02 mol L^{-1}) in 1000 ml of water, adjust the pH to 4.5 with ammonia or glacial acetic acid.(solvent B), Acetonitrile]. The contents of the mobile phase were mixed in the ratio of ammonium dihydrogen phosphate buffer: acetonitrile (65:35) and filtered before use through a 0.45 µm membrane filter, sonicated and pumped from the solvent reservoir to the column at a flow rate of 1 mL min^{-1}.

2.4. Standard solution

Standard stock solutions 1 mg mL^{-1} of rosiglitazone and metformin were prepared in methanol and further diluted in mobile phase. The working standard solutions were prepared in mobile phase to contain mixture of rosiglitazone and metformin in over the linearity range from 12 –32 µg mL^{-1} and 20- 70 µg mL^{-1}.

2.5. Assay in formulation

Twenty tablets each containing and their average weight was calculated. The tablet were crushed to furnish a homogeneous powder and a quantity equivalent to one tablet were weighed in to a 100 mL volumetric flask, dissolve in methanol, sonicated for about 15 min and then made up to volume with mobile phase. The solution was stirred for 10 min using a magnetic stirrer and filtered into a 100 mL volumetric flask through 0.45 µm membrane filter. The residue was washed 3 times with 10 mL of mobile phase, and then the volume was completed to 100 mL with the same solvent. Further add mobile phase to obtain an expected concentration of 100µg mL^{-1} metformin and 4 µg mL^{-1} rosiglitazone. All determinations were conducted in triplicate.

3. Results and Discussions

The proposed HPLC method required fewer reagents and materials and it is simple and less time consuming. This method could be used in quality control test in pharmaceutical industries. The chromatogram of rosiglitazone and metformin were shown in (Fig.1). There was clear resolution between rosiglitazone and metformin with retention time of 7.19±0.044 and 5.57±0.038 minutes, respectively.

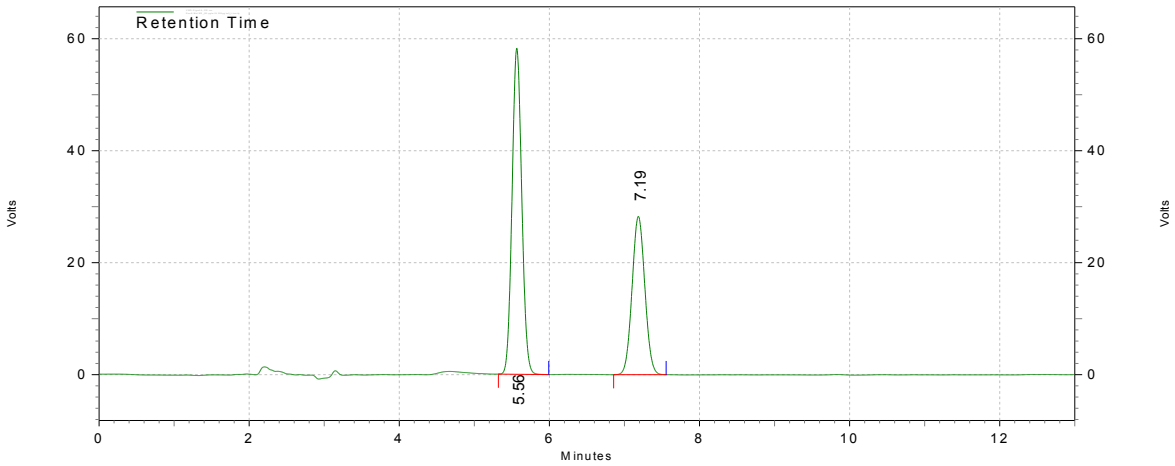


Fig. 2. Typical chromatogram showing metformin and rosiglitazone

3.1. Linearity

The response was determined to be linear over the range of 12 µg mL^{-1} to 32 µg mL^{-1} (12, 16, 20, 24, 28, 32) for rosiglitazone and 20- 70 µg mL^{-1} (20, 30, 40, 50, 60, 70) for metformin. The solutions were injected into HPLC system. Each of the concentration was injected in triplicate to get reproduciable response. The run time was 13 min and the peak areas were measured (Table 1 & 2). The calibration curve was plotted as concentration of the respective drug versus the response at each level. The purposed method was evaluated by its correlation coefficient and intercept value calculated by statistical study.They were represented by the linear regression equation (Fig 2 and 3 calibration curve).

$Y_{Rosiglitazone} = 545663x - 791146$ Coefficient of correlation (r^2) value = 0.999

$Y_{Metformin} = 2310186x + 338597$ Coefficient of correlation (r^2) value =0.9982

Table 1. For Peak Area of Rosiglitazone

Concentration (µg mL^{-1})	12	16	20	24	28	32
Replicate 1	5641340	8050019	10118867	12288158	14371991	16857253
Replicate 2	5616147	8019480	10310784	12296490	14243722	16527580
Replicate 3	5660735	8063209	10280031	12354696	14268855	16872200
Avg	5639407	8044236	10236561	12313115	14294856	16752344
SD	22356.74	22430.75	103079	36250.67	67972.59	194795
RSD	0.396438	0.278843	1.006969	0.294407	0.475504	1.162793

Table 2. Peak area of Metformin

Concentration (µg mL^{-1})	20	30	40	50	60	70
Replicate 1	8804979	12735975	16046569	19228421	22445385	26258417
Replicate 2	8790742	12791380	16042475	19122360	22262776	26254189
Replicate 3	8912430	12825416	16007754	19037240	22127453	26152968
Avg	8836050	12784257	16032266	19129340	22278538	26221858
SD	66528.66	45143.95	21326.48	95781.46	159551	59697.93
RSD	0.752923	0.353121	0.133022	0.500704	0.716165	0.227665

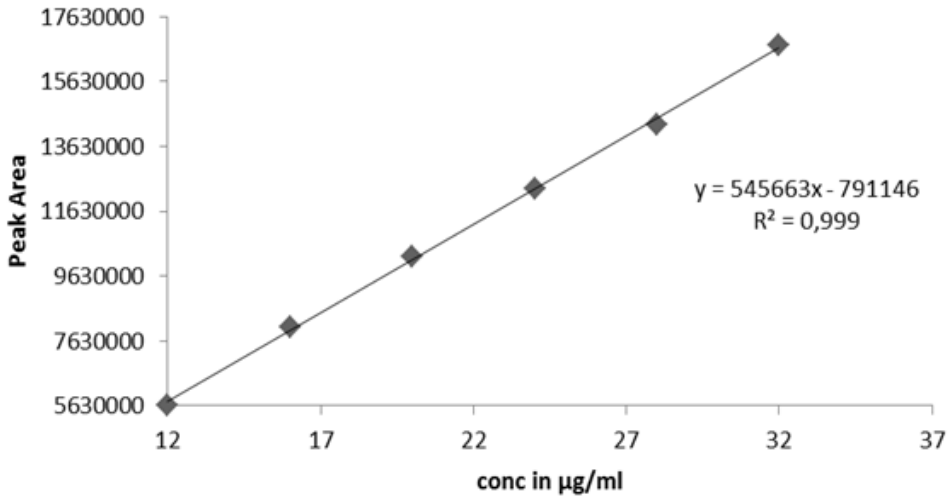


Fig. 3. Calibration curve for Rosiglitazone

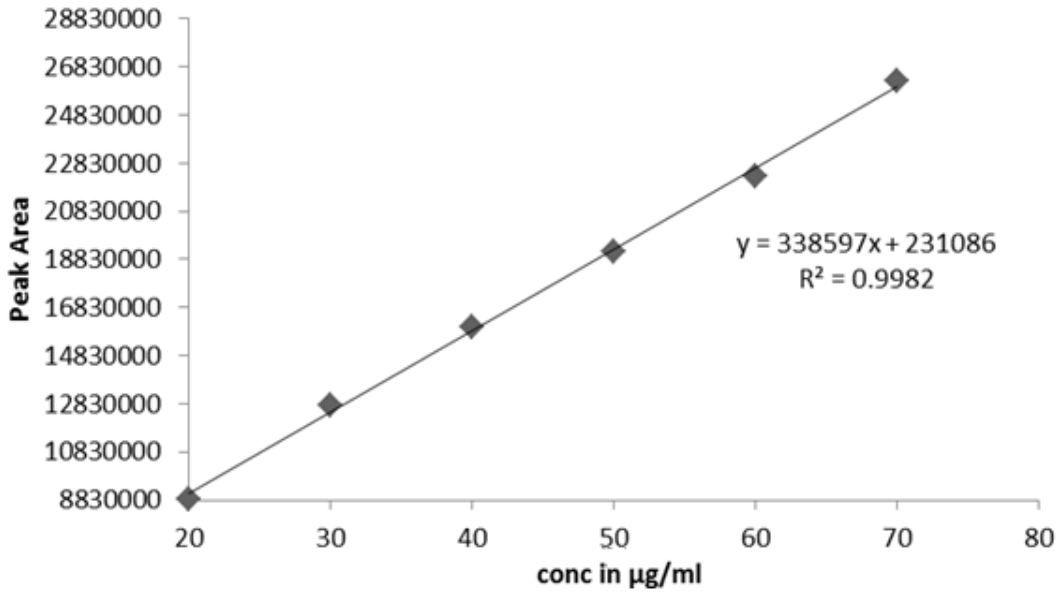


Fig. 4. Calibration curve for Metformin

3.2. Accuracy

The accuracy is the closeness of the measured value to the true value for the sample. Accuracy was found out by recovery study from prepared solution (three replicates) with standard solution, of the label claim. Aliquots of 1 mL, 2 mL and 4 mL of sample drug solution were pipetted into each of three volumetric flasks. To this 0.8 ml of rosiglitazone standard drug solution of 100 µg mL^{-1} was added to each volumetric flask respectively. To this 1 mL of metformin standard drug solution of 100µg mL^{-1} was added to each volumetric flask respectively. The volume was made up to 10 mL with mobile phase. 20 µL of each solution was injected and chromatograms were recorded. The range was found between 97.72 to 100.43 % respectively. The values of recovery justify the accuracy of the method. The % recovery values were obtained within the standard limit which confirms that the method is accurate and free from any positive or negative interference of the excipients (Table 3).

Table 3. Result of recovery studies

Sample	Concentration (µg mL^{-1})	Std addition in (µg mL^{-1})	Total Concentration found (µg mL^{-1})*	Recovery, %
ROSI	8	4	11.83	98.59
MET	10	10	19.52	97.72
ROSI	8	8	16.00	100.03
MET	10	20	30.13	100.46
ROSI	8	16	24.10	100.43
MET	10	40	49.94	99.88
Mean ± SD	ROSI		99.69±0.966	
	MET		99.35±1.180	

3.3. Limit of Detection and Quantification

Limit of detection is determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be reliably detected.

The detection limit (LOD) and quantitation limit (LOQ) may be expressed as:

L.O.D. = 3.3(SD/S)

L.O.Q. = 10(SD/S)

Where, SD = Standard deviation of the response, S = Slope of the calibration curve

The slope S may be estimated from the calibration curve of the analyte.

The LOD was found to be 1.100 μg mL^{-1} and 0.725 μg mL^{-1} and LOQ was found to be 3.66 μg mL^{-1} and 2.41 μg mL^{-1} for metformin and rosiglitazone respectively which represents that sensitivity of the method is high.

3.4. Precision

Repeatability involves analysis of replicates by the analyst using the same equipment and method and conducting the precision study over short period of time while reproducibility involves precision study at different occasions, different laboratories, and different batch of reagent, different analysts, and different equipments. The repeatability study which was conducted on the solution having the concentration of about 18 μg mL^{-1} for rosiglitazone and 32 μg mL^{-1} for metformin (n =5) showed a RSD of 0.526% for rosiglitazone and 0.369% for metformin. It was concluded that the analytical technique showed good repeatability (Table 4).

Table 4. Results of repeatability analysis

Sample	Concentration (μg mL^{-1})	Peak Area (μV*sec)	Mean±SD	%RSD
ROSI	18	8542812	8554098 ± 31618.82	0.369
		8532224		
		8523084		
		8599827		
		8572541		
MET	32	13322996	1328495 ± 69744	0.520
		13251750		
		13145545		
		13308122		
		13264064		

3.5. Reproducibility and Ruggedness

The ruggedness of an analytical method is determined by analysis of aliquots from homogenous lots by different analysts using operational and environmental conditions that may differ but are still within the specified parameters of the assay. The assay was performed in different condition, different analyst, and different dates (Table 5).

Table 5. Results of reproducibility

Parameter	Result observed	
	ROSI	MET
Average Percentage Recovery	100.20%	100.21%
SD between set of analysis on same date	0.451	0.849
SD between set of analysis on different date	0.832	1.634
RSD between set of analysis on same date	0.451%	0.849%
RSD between set of analysis on different date	0.83%	1.63%

3.6. Robustness

The robustness of the method was determined by delibrate changes in the method like alteration in pH of the mobile phase, percentage organic content, changes in the wavelength. The robustness of the method shows that there were no marked changes in the chromatographic parameters, which demonstrates that the method developed is robust.

3.7. Specificity

The selectivity of an analytical method is its ability to measure accurately and specifically the analyte of interest in the presence of components that may be expected to be present in the sample matrix. If an analytical procedure is able to separate and resolve the various components of a mixture and detect the analyte qualitatively the method is called selective. It has been observed that there are no peaks of diluents and placebo at main peak's. Hence, the chromatographic system used for the estimation of rosiglitazone and metformin is very selective and specific .Specificity studies indicating that the excipients did not interfere with the analysis. For demonstrating the specificity of the method for drug formulation the drug was spiked and the representative chromatogram (Fig.5)

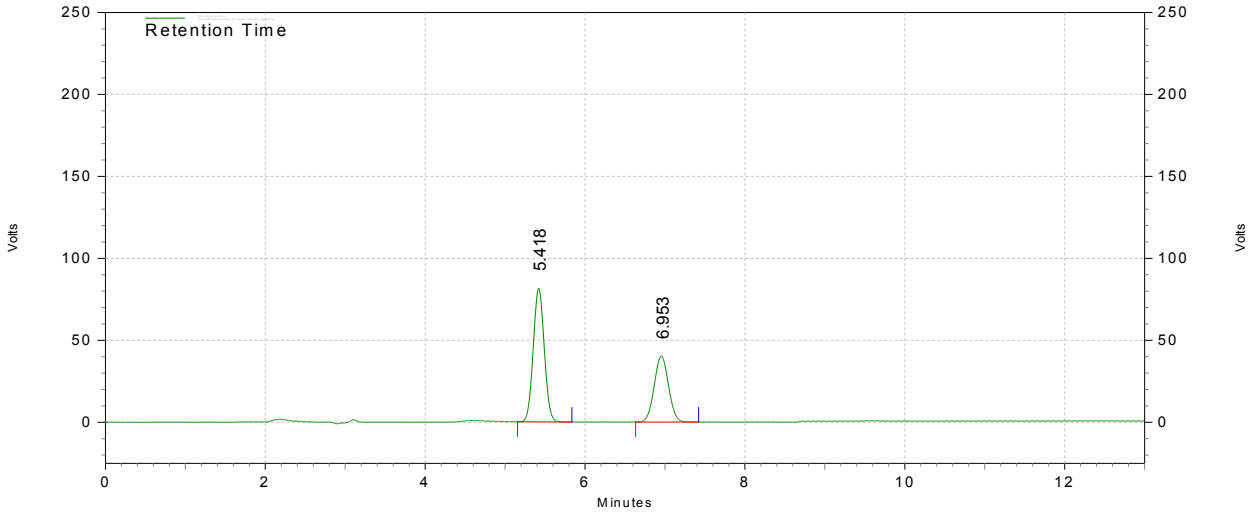


Fig.5. Chromatogram showing Specificity

3.8. System Suitability

A binary solution of 32 µg $mL^{-1}$of rosiglitazone and 70 µg mL^{-1} of metformin (in triplicate) was prepared and same was injected, then the system suitability parameters like resolution factor (R_s), tailing factor (T_f) and theortical plates (N) were calculated and recorded in Table 6. The values for system suitability parameters showed feasibility of this method for routine pharmaceutical application

Table 6. Results of system suitability parameters

Parameters	Data obtained	
	ROSI	MET
Number of theortical plates (N	3524	4477
Tailing factor (T_f)	1.16	1.01
Resolution (R_s)	4.35	

4. Conclusion

The proposed RP-HPLC method is found to be simple, accurate, precise, linear, and specific for quantitative estimation of rosiglitazone and metformin in bulk and its formulation. The proposed RP-HPLC method is cost effective and less time consuming. The values for system suitability parameters showed feasibility of this method for routine pharmaceutical application. Hence the present HPLC method is suitable for routine assay of rosiglitazone and metformin in raw materials and in pharmaceutical formulations in the quality control laboratories.

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